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RELATIONSHIP BETWEEN SERUM REACTIONS *

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It has been generally accepted that there is no relation between serum reactions in so far as the numeric values of the titers are concerned; that, in other words, the serologic qualities of an animal are so irregular in their correlationships that no conclusion concerning one serum reaction can be arrived at by knowledge of another. However, in the course of routine work with complement-fixation I often have had the impression that rabbit sera which give high hemolytic titers show also high precipitins (the latter being due to small quantities of serum adhering to the sheep corpuscles), and as the question involved is not only of interest but of great importance, I have found it advisable to make systematic tests. Furthermore, one of our chief problems here is to find an easier method of testing the potency of hog-cholera immune serum, a question intimately connected with the relationship of serum reactions.

There are two kinds of reactions which present the greatest probability of a connection: that in which the antigen is uniform tho the antibodies differ, and that in which the antigen is multiform tho the titrated antibodies are of the same kind. To test the first class of reactions I selected a blood-corpuscle emulsion as antigen which injected into an animal produces several different antibodies, 2 of which, agglutinins and hemolysins, are easily titrated.

Experiment 1.—Five rabbits were injected intraperitoneally with 5 c.c. of a 5% emulsion of sheep corpuscles each. The injections were repeated on the 5th and 10th days. After 5 days blood was drawn from these animals and the agglutinins and hemolysins titrated. All the agglutinins were titrated after the following formula:

Tube	Salt Solution	Serum	Emulsion	Dilution
1		0.5 c.c. (10%)	0.5 c.c.	1:20
2	0.5 c.c.	0.5 c.c. of Tube 1	0.5 c.c.	1:40
3	0.5 c.c.	0.5 c.c. of Tube 2	0.5 c.c.	1:80
4	0.5 c.c.	0.5 c.c. of Tube 3	0.5 c.c.	1:160
5	0.5 c.c.	0.5 c.c. of Tube 4	0.5 c.c.	1:320
6	0.5 c.c.	0.5 c.c. of Tube 5	0.5 c.c.	1:640
7	0.5 c.c.	0.5 c.c. of Tube 6	0.5 c.c.	1:1289
8	0.5 c.c.	0.5 c.c. of Tube 7	0.5 c.c.	1:2560

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The titration of hemolysins employed 0.5 c.c. serum dilution, 1 c.c. salt solution, 0.5 c.c. complement (5% guinea-pig serum), and 0.5 c.c. blood-corpuscle emulsion (4%). The dilutions of serum used were 1: 100, 1: 500, 1: 1000, 1: 2000, 1: 3000, 1: 5000.

The results of this first experiment were as follows:

Rabbit	Agglutinins	Hemolysins
1	1:160	1:1000
2	1:320	1:100
3	1:160	1:3000
4	1:1280	1:100
5	1:320	1:500

Apparently there is no relation in strength between the two reactions in these five cases.

Experiment 1 was repeated with 5 other rabbits, one of which died before the blood could be taken.

Rabbit	Agglutinins	Hemolysins
1	1:320	1:600
2	1:1280	1:800
3	1:640	1:2000
4	1:640	1:500

Here too the incongruity of the two reactions is apparent.

The same experiment with guinea-pigs gave the following titers:

Guinea-Pig	Agglutinins	Hemolysins
1	1:2560	1:2000
2	1:640	Below 1:100
3	1:2560	1:1000
4	1:1280	1:2000
5	1:640	1:100

In this case the two lowest agglutinating titers correspond to the two lowest hemolytic titers.

Experiment 2.—This experiment, complicated by the simultaneous use of 3 blood emulsions at the same time, belongs partly to the second class of reactions. An emulsion consisting of equal amounts of horse, sheep, and pig corpuscles was used. It was intravenously injected in doses of 2 c.c., and the treatment after 5 days was repeated. The reactions may be compared in view of the first class of reactions as follows:

Rabbit	Agglutinins			Hemolysins		
	Horse Corpuscles	Pig Corpuscles	Sheep Corpuscles	Horse Corpuscles	Pig Corpuscles	Sheep Corpuscles
1	1:5120	1:320	1:320	1:100	Below 1:100	1:1000
2	1:5120	1:640	1:320	1:1000	Below 1:100	1:1000
3	1:2560	1:320	1:640	1:100	Below 1:100	1:5000
4	1:2560	1:160	1:40	Below 1:100	1:100	1:1000
5	1:1280	1:1280	1:320	1:100	Below 1:100	1:3000

These three groups show only a very slight parallelism between the two reactions. The comparison in the sense of the second group can be made directly on the tabulations. The arrangement of the sera according to the numeric values of their titers is as follows:

Agglutinins		
Horse Corpuscles	Pig Corpuscles	Sheep Corpuscles
No. 1, No. 2,	No. 5,	No. 3,
No. 3, No. 4,	No. 2,	No. 1, No. 2, No. 5,
No. 5,	No. 1, No. 3,	No. 4.
	No. 4.	

Hemolysins		
Horse Corpuscles	Pig Corpuscles	Sheep Corpuscles
No. 2,	No. 4,	No. 3,
No. 1, No. 3, No. 5,	No. 3, No. 5,	No. 5,
No. 4.	No. 1, No. 2.	No. 1, No. 2, No. 4.

The results of the comparisons are apparently negative.

Experiment 2 was repeated, intraperitoneal injections of 5 c.c. of mixed emulsion being used, with 5 other rabbits, 2 of which were lost. Below are the titers of the three remaining:

Rabbit	Agglutinins			Hemolysins		
	Horse Corpuscles	Pig Corpuscles	Sheep Corpuscles	Horse Corpuscles	Pig Corpuscles	Sheep Corpuscles
1	1:640	1:160	1:640	1:500	1:100	1:3000
2	1:1280	1:1280	1:320	1:500	1:100	1:1000
3	1:320	1:160	1:40	1:100	1:1000	1:3000

Experiment 3.—In this experiment the rabbits of the first experiment were employed. They were injected 3 times at intervals of 5 days with an emulsion of B. paratyphosus B. This was prepared by washing off 24-hour-old agar cultures with 10 c.c. of normal salt solution and heating the emulsion for 45 minutes at 65 C. Seven days after the last injection these rabbit sera were titrated for agglutinins. The results compared with the previously ascertained hemagglutinin titers are as follows:

Rabbit	Agglutinins for B. paratyphosus B	Agglutinins for Sheep Corpuscles
1	1:1280	1:160
2	1:5120	1:320
3	1:2560	1:160
4	1:320	1:1280
5	1:1280	1:320

There is no relation between these two titers.

Experiment 4.—The agglutinin titers against 3 different antigens were compared. Emulsions of B. coli., B. subtilis, and B. pyocyaneus were used on 5 rabbits. The technic was the same as in Experiment 3, except that the emulsions were counted and their concentrations equalized.

Rabbit	Agglutinins		
	B. Coli	B. Subtilis	B. Pyocyaneus
1	1:320	1:80	1:40
2	1:160	1:160	Below 1:20
3	1:1280	1:640	1:80
4	1:320	Below 1:20	1:20
5	1:320	1:1280	1:40

Serum 3 excepted, there is hardly any parallelism between the titers. The sera, arranged according to the titration, are found as follows:

For B. coli	For B. subtilis	For B. pyocyaneus
No. 3	No. 5	No. 3
No. 4	No. 3	No. 5
No. 5, No. 1	No. 2	No. 1
No. 2	No. 1	No. 4
	No. 4	No. 2

The repetition of Experiment 4 gave the following results:

Rabbit	Agglutinins		
	B. Coli	B. Subtilis	B. Pyocyaneus
1	1:640	1:320	1:80
2	1:160	1:160	1:640
3	1:640	1:160	1:640

Rabbits 4 and 5 were accidentally lost. The comparison of these tabulations gives a negative result.

Experiments 5 and 6.—Seroprecipitins against 3 different antigens were compared. Five rabbits were injected 3 times intravenously at intervals of 5 days. An equal mixture of horse, pig, and sheep serum was used in doses of 6 c.c. For the titration different dilutions of horse, pig, or sheep serum were underlaid with 0.1 c.c. of the rabbit serum to be titrated.

Rabbit	Seroprecipitins		
	Horse	Pig	Sheep
1	1:1000	1:1000	1:3000
2	Below 1:100	1:100	1:100
3	1:100	1:1000	1:1000
4	1:1000	1:8000	1:5000
5	1:5000	1:5000	1:3000

With the exception of the titer of Rabbit Serum 4 for horse serum, the coincidence of these titers is manifest. Below the sera are arranged according to their titration:

For Horse Serum	For Pig Serum	For Sheep Serum
No. 5	No. 4	No. 4
No. 4, No. 1	No. 5	No. 5
No. 3	No. 3, No. 1	No. 1
No. 2	No. 2	No. 3
		No. 2

This experiment was repeated with the following results:

Rabbit	Seroprecipitins		
	Horse	Pig	Sheep
1	1:1000	1:3000	1:5000
2	1:500	1:1000	1:1000
3	1:500	1:500	1:500
4	1:1000	1:1000	1:1000
5	1:1000	1:1000	1:3000

In this tabulation too a parallelism of the titer values cannot be denied.

SUMMARY

There was no coincidence between the titers of hemagglutinins and hemolysins against the same antigen.

There was none between the titers of hemagglutinins against different antigens or between the titers of hemolysins against different antigens.

There was none between titers of agglutinins for bacteria, when 3 different antigens were employed, namely, B. coli, B. subtilis, B. pyocyaneus.

In 2 tests, each of 5 rabbits, seroprecipitins were produced rather evenly, 3 different antigens being used.